

Synthesis and Radiolabeling of Heterocyclic Food Mutagens

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The imidazoquinoline and imidazoquinoxaline food mutagens found in cooked meat are being synthesized by unambiguous methods that allow for the preparation of sufficient quantities of material for biological studies. These methods avoid difficult separations of regioisomeric mixtures of products and are designed to allow incorporation of specific high level tritium labeling.

Synthesis of IQ and MeIQ

In 1980, two highly mutagenic heterocyclic amines were isolated from cooked meats, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (methyl IQ, MeIQ) (1,2). Both of these compounds have been synthesized, but by somewhat limited methods (3-5). Their structures are shown in Figure 1.

We have recently completed the synthesis of IQ and MeIQ (6). In devising our syntheses we had three primary objectives in mind: (1) the syntheses should avoid regioisomeric problems with their concomitant structural ambiguities and chromatographic separations; (2) the methods should permit the preparation of large (~10 g) amounts of final product to support the necessary biological studies; and (3) the syntheses should be adaptable to the preparation of radiolabeled product which contains label that is both site-specific and of high specific activity to facilitate small scale and low efficiency biological studies.

All of these objectives have been met, and the syntheses are described in Figures 2 through 6.

The synthesis of IQ is shown in Figures 2 and 3. It proceeds from 6-bromoquinoline. The bromine is then displaced and the methylamino group unambiguously introduced to give 6-methylamino-5-nitroquinoline. This is then reduced to the diamino compound, a key intermediate. To close the imidazole ring, either of two methods are used. If ammonium formate is used, the 2-amino

group is then introduced via the C-2 anion and reaction with phenylazide. If cyanogen bromide is used, the 2-amino group accompanies ring closure.

The synthesis of tritium-labeled IQ is shown in Figure 3. For this purpose, IQ is brominated to give the 5-bromo compound as the only isomer. The bromine atom is then replaced by tritium using tritium gas with a palladium catalyst. Thus IQ is available with specific activity up to 17 Ci/mmol. This synthesis can be easily adapted for the preparation of 10 gram quantities of IQ.

The synthesis of MeIQ is shown in Figures 4, 5, and 6. It begins with the tosyl derivative of the known 2-bromo-3-methylaniline (7). This is nitrated and the desired nitro isomer is obtained after the only isomer separation in the sequence. The direction of quinoline ring formation is controlled by the bromo substituent. Reduction and methylation then gives the required methylaminoquinoline (Fig. 4).

Nitration now gives the nitramine which on warming in acid rearranges to the nitroquinoline (Fig. 5). Reduction with hydrazine and Raney nickel proceeds to the diamine with retention of the bromo substituent. As in the case of IQ, reaction with BrCN closes the imidazole ring and forms 5-bromoMeIQ. To prepare MeIQ the bromine is removed by hydrogenolysis with $^1\text{H}_2$; to prepare [$5\text{-}^3\text{H}_1$]-MeIQ, hydrogenolysis is carried out with $^3\text{H}_2$ (Fig. 6). This synthesis also results in significant (~10 g) quantities of MeIQ, and the tritium label is site-specific (C-5) and of high specific activity. Should a ^{14}C label be desired, this can be obtained in both syntheses by using Br^{14}CN . This would put a ^{14}C at position 2.

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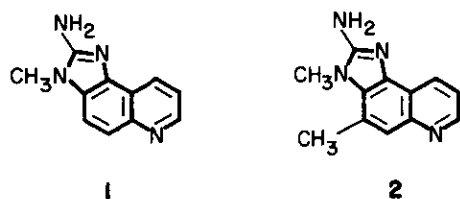


FIGURE 1. Structures of IQ and MeIQ.

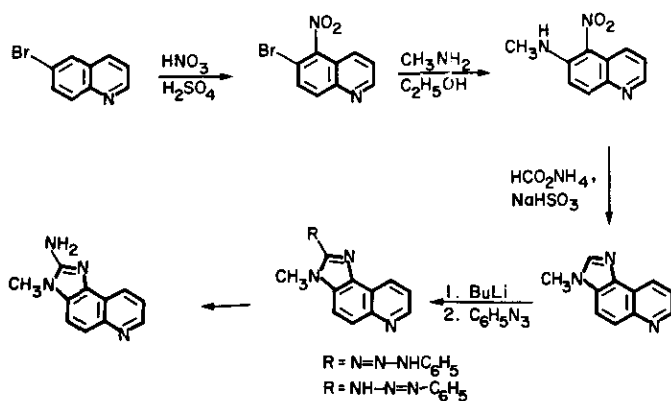
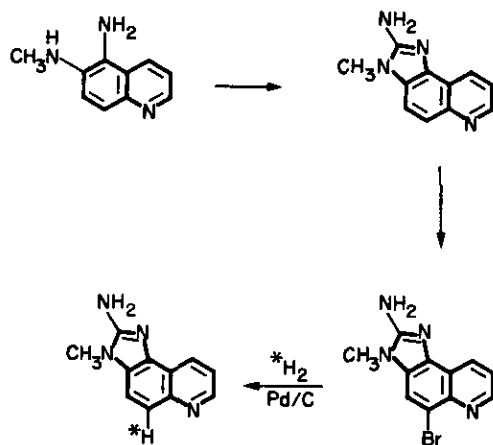


FIGURE 2. Synthesis of IQ.

FIGURE 3. Synthesis of 3H -IQ.

Synthesis of the Imidazoquinoxalines

With the same objectives in mind, namely, syntheses that are regiochemically unambiguous, can be carried out on a large scale, and allow the introduction of radiolabel, we have planned a new approach for the synthesis of the imidazoquinoxalines. The specific compounds we are synthesizing are the following six: 8-methyl and 7-methyl IQx; 4,8 and 5,8-dimethyl IQx; and

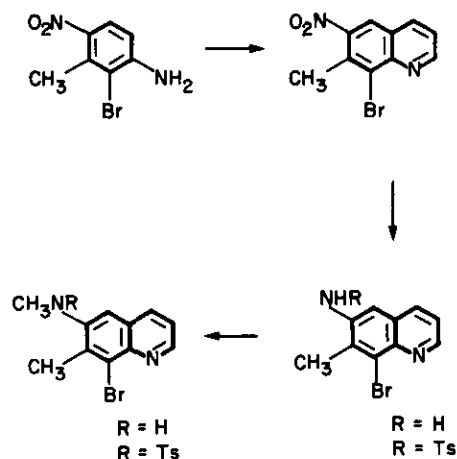


FIGURE 4. Synthesis of 8-bromo-7-methyl-6-methylaminoquinolinol.

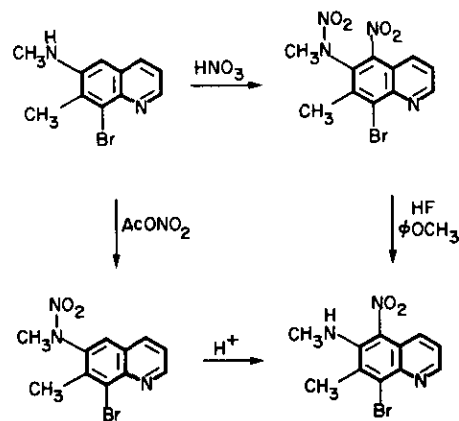


FIGURE 5. Nitration studies.

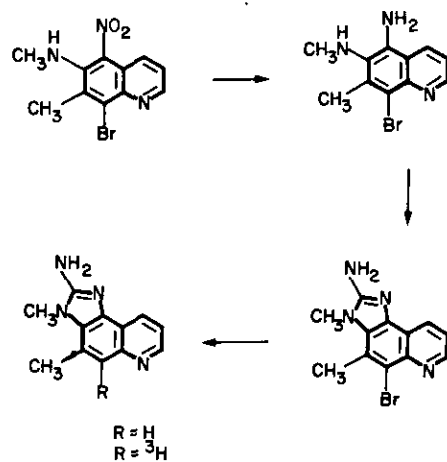


FIGURE 6. Synthesis of MeIQ.

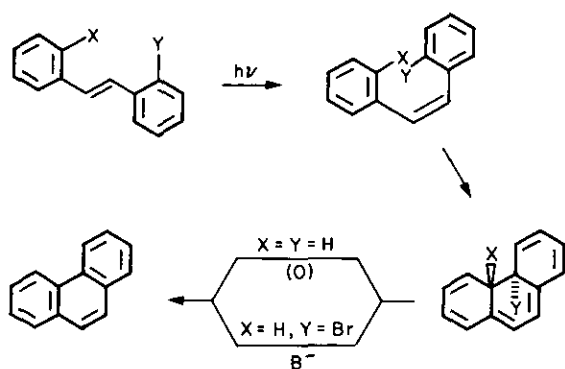


FIGURE 7. Synthesis of angular aromatic tricyclic compounds.

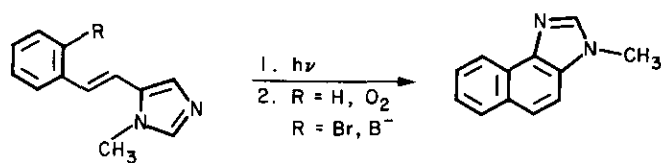


FIGURE 8. Photocyclization of a styrylimidazole to an imidazonaphthalene.

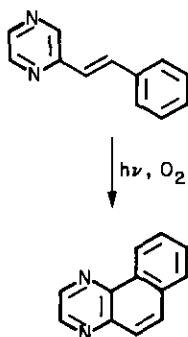


FIGURE 9. Photocyclization of a styrylpyrazine to a diazaphenanthrene.

4,7 and 5,7-dimethyl IQx.

Some of these imidazoquinoxalines are powerful mutagens recently isolated from fried beef (8). A synthesis has been developed (9), but the process presents two potential regioisomer problems. In one step, condensation of a diamine with methylglyoxal did indeed give equal quantities of both regioisomers. In the other instance, nitration apparently gave only one isomer. However, since yields were not reported, the effectiveness of this synthesis remains in doubt.

We have patterned our synthetic strategy for the preparation of these angular tricyclic molecules on the methods that have proved most successful for the parent tricyclic phenanthrene (Fig. 7). These methods all depend on an intermediate stilbene in which the central ring is formed by a number of cyclization processes. The

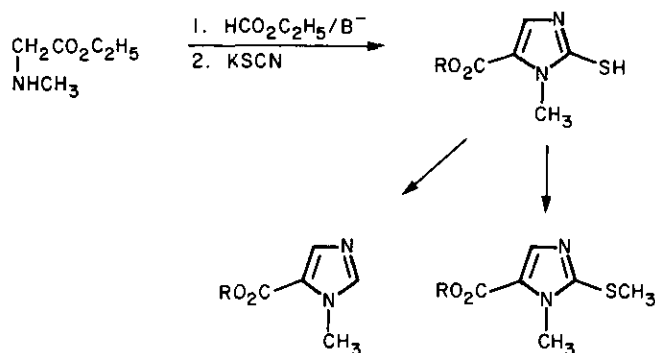


FIGURE 10. Synthesis of 1,5-substituted-N-methylimidazoles.

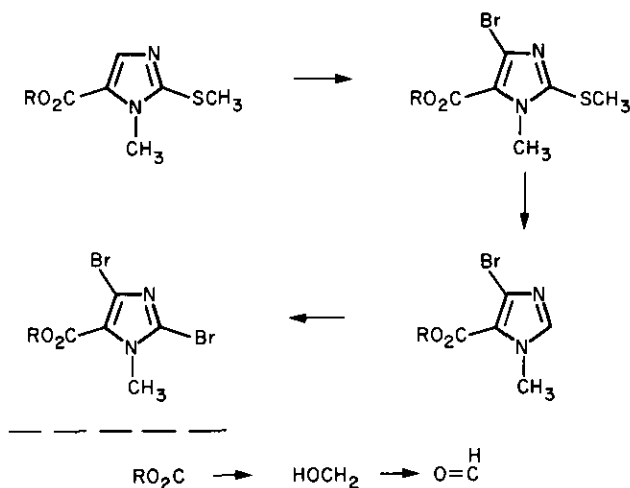


FIGURE 11. Synthesis of imidazole derivatives.

most versatile cyclization method is photocyclization, and this process has been recently comprehensively reviewed (10). The overwhelming number of examples are for substituted phenanthrenes, as illustrated in Figure 7. Final aromatization is achieved by removal of H_2 by oxidation or removal of HBr with base.

Although application of this method to heterocycles is much more limited, sufficient evidence exists to indicate its potential. Thus the styrylimidazole has been successfully photocyclized to the imidazonaphthalene (13) as shown in Figure 8. Also, a styrylpyrazine has been photocyclized to the corresponding diazaphenanthrene (14) shown in Figure 9. Both of these reactions are oxidative photocyclization in which two hydrogens are removed from the dihydro intermediate. Alternative processes are also available in which a bromine atom is situated at one of the cyclization positions and aromatization is accomplished by dehydrobromination with alkali. We have successfully carried out cyclizations by both oxidative and dehydrobrominative procedures. Thus the literature clearly documents that both "half" reactions proceed well. Both the imidazole

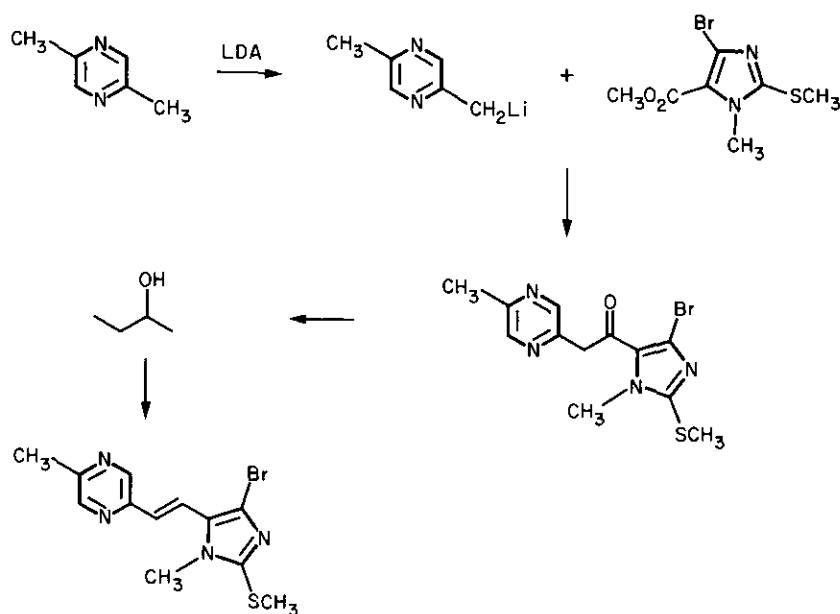


FIGURE 12. Synthesis of an imidazolyl pyrazinylethylene.

and pyrazine rings will undergo this photocyclization reaction with a benzene ring. What remained to be demonstrated, and what we have now accomplished in the laboratory, is the photocyclization of imidazoles with pyrazines to yield imidazoquinoxalines.

To proceed with our syntheses, a number of N-methylimidazoles were required of unambiguous regiochemistry. For this purpose we adapted the synthetic method of Jones (13) shown in Figure 10. Large amounts of the imidazole intermediates are thus available, and these are readily transformed into the required further substitution products shown in Figure 11.

Using these imidazoles and the anion of the readily available 2,5-dimethylpyrazine, we carried out a condensation to obtain the ketone. The ketone was reduced to the alcohol which was dehydrated to the disubstituted ethylene. This ethylene corresponds to the previous styrenes and its synthesis is shown in Figure 12.

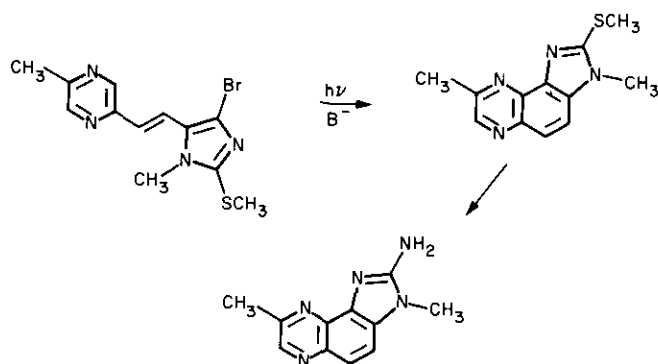


FIGURE 13. Synthesis of methyl IQx.

With the imidazolyl pyrazinyl ethylene in hand, we proceeded to the photocyclization. Although both oxidative and dehydrobrominative photocyclizations led to imidazoquinoxalines, the dehydrobrominative method gave better yields. The final step, replacement of the SCH₃ group by amino, was successful, but the yields were poor. Improvements are being sought in variations of the SCH₃ group. This synthesis of MeIQx is illustrated in Figure 13.

To introduce the methyl group needed for the 5-methyl analog, we returned to the intermediate ketone. The enolate ion was formed with sodium hydride then alkylated with methyl iodide. Reduction to the alcohol, elimination, and dehydrobrominative photocyclization

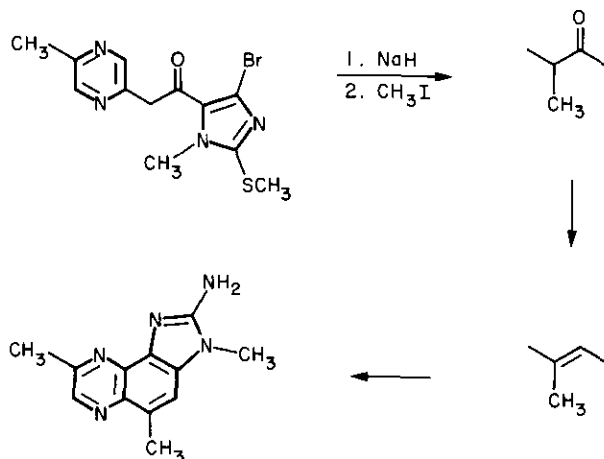


FIGURE 14. Synthesis of 5,8-dimethyl IQx.

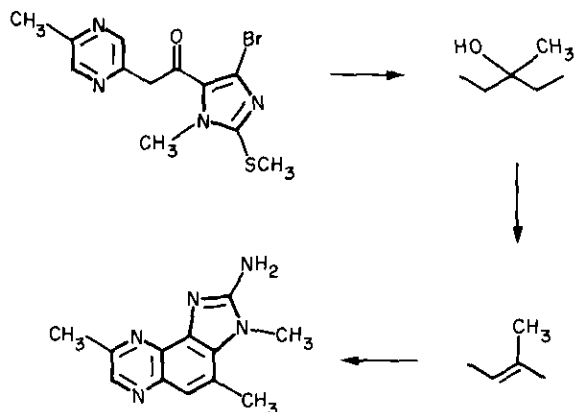


FIGURE 15. Synthesis of 4,8-dimethyl IQx.

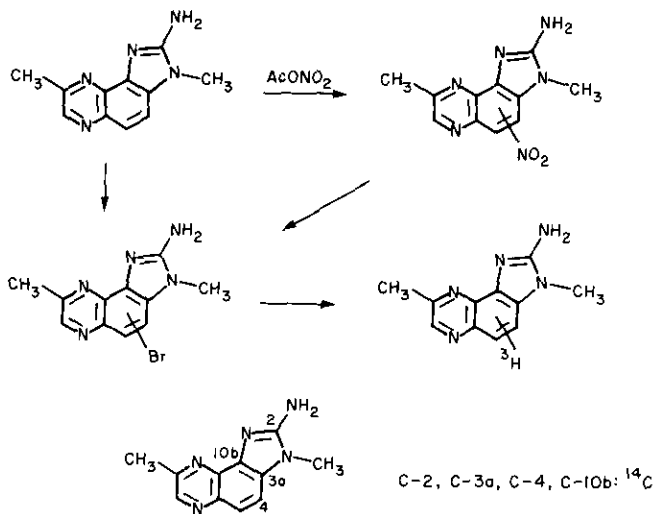


FIGURE 16. Introduction of radiolabel into the imidazoquinoxalines.

then led to 5,8-dimethylIQx. This sequence is given in Figure 14.

The corresponding 4-methyl analog is prepared by the sequence shown in Figure 15. Again, the ketone is the key intermediate. With the methyl Grignard reagent, this ketone is converted to tertiary alcohol. Dehydration

gives the substituted stilbene which is dehydrobrominatively photocyclized. The corresponding 7-methyl analogs are made by the directly parallel route starting with 2,6-dimethylpyrazine.

The opportunities for introducing radiolabels, both ^{14}C and ^3H , are presented in Figure 16. To introduce the ^3H label, the nucleus will be nitrated, and the nitro group will be replaced by bromo via the amino derivative. Hydrogenolysis will then replace Br by ^3H . The possibilities for introducing ^{14}C are many by our method of synthesis, and a ^{14}C label can be present at C-2, C-3a, C-4, and C-10b.

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REFERENCES

1. Spingarn, N. E., Kasai, H., Vuolo, L. L., Nishimura, S., Yamaizumi, Z., Sugimura, T., Matsushima, T., and Weisberger, J. H. *Cancer Letters* 9: 177-000 (1980).
2. Kasai, H., Nishimura, S., Wakabayashi, K., Nagao, M., and Sugimura, T. *Proc. Japan Acad.* 56B: 383-000 (1980).
3. Kasai, H., Yamaizumi, Z., Nishimura, S., Wakabayashi, K., Nagao, M., Sugimura, T., Spingarn, N. E., Weisburger, J. H., Yokoyama, S., and Miyazawa, T. *J. Chem. Soc. Perkin I*, 2290-0000 (1981).
4. Lee, C.-S., Hashimoto, Y., Shudo, K., and Okamoto, T. *Chem. Pharm. Bull.* 30: 1857-0000 (1982).
5. Kasai, H., and Nishimura, S. *Bull. Chem. Soc. Japan* 55: 2233-0000 (1982).
6. Waterhouse, A. L., and Rapoport, H. J. *Labeled Compounds Radiopharm.* 22: 201-216 (1984).
7. Newman, M. S., and Kanan, R. J. *J. Org. Chem.* 41: 3356-0000 (1976).
8. Kasai, H., Yamaizumi, Z., Shiomi, T., Yokoyama, S., Miyazawa, T., Wakabayashi, K., Nagao, M., Sugimura, T., and Nishimura, S. *Chem. Letters* 485-000 (1981).
9. Kasai, H., Shiomi, T., Sugimura, T., and Nishimura, S. *Chem. Letters* 675-000 (1981).
10. Mallory, F. B., Mallory, C. W. *Photocyclization of stilbenes and related molecules*. In: *Organic Reactions* Vol. 30, Wiley, New York, 1984.
11. Lundgren, G., Stensio, K. E., and Wahlberg, K. J. *Heterocyclic Chem.* 17: 679-000 (1980).
12. Perkampus, J. H., and Bluhm, T. *Tetrahedron* 28: 2099-0000 (1972).
13. Jones, R. G. *J. Am. Chem. Soc.* 71: 644-000 (1949).